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Long-term surveillance of invasive group A streptococcal disease in The Netherlands, 1994–2003

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ABSTRACT

A nationwide laboratory-based surveillance study of invasive group A streptococcal (GAS) infections was conducted in The Netherlands from May 1994 until December 2003 (average population during this period was 15 729 704). Microbiologically invasive isolates were obtained from 1504 patients, with most (70%) isolates cultured from blood. There was a clear seasonal pattern in invasive streptococcal infections, with an estimated annual incidence that peaked in 1996 (4.0 cases/100 000 individuals/year) and was at its lowest in 1999 (2.0 cases/100 000 individuals/year). Twenty-eight different M-types were identified, of which the most frequent were M1 (339/1504, 23%), M3 (187/1504, 12%), M89 (174/1504, 12%), M28 (164/1504, 11%), M12 (109/1504, 7%) and M6 (55/1504, 4%). There was a high degree of variation in the relative annual contributions of the predominant M-types, but variations in M1 and M3 combined correlated with overall changes in the annual incidence. The contribution of the patient group aged ≥56 years to all cases of invasive GAS disease increased during the study period, whereas that of the group aged 0–20 years decreased. A peak in the incidence of invasive GAS disease among the patient group aged 30–34 years did not vary during the study period, indicating that the high incidence of invasive GAS disease in this age group was age-specific rather than cohort-related.

Keywords Group A streptococcal infections, invasive streptococcal infections, streptococcal infections, surveillance, The Netherlands

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INTRODUCTION

Group A streptococci continue to represent a major cause of infectious disease-linked morbidity and mortality worldwide [1]. Although most group A streptococcal (GAS) infections have a mild clinical course, a certain proportion progress to severe invasive disease, which can be complicated by the development of streptococcal toxic shock-like syndrome (TSS) [2,3]. A remarkable feature of invasive GAS disease is its epidemiology. After more than half a century of decreasing morbidity and mortality, a resurgence and persistence of severe invasive GAS infections has been noted since the mid-1980s [1–6]. The cause for this resurgence of streptococcal infections remains enigmatic, and many hypotheses have emerged, focusing either on impaired host defences or an increase in the virulence of the bacterium itself [7–9].

Previous studies have shown that immunity to proteins and toxins that attach to the cell wall may play a role in protection against severe invasive disease [10]. The lack of protective anti-toxin antibodies is known to be age-dependent [11]. Furthermore, a number of laboratory-based studies have suggested that the recent appearance of serotypes with increased virulence may provide an explanation for the observed changes in GAS epidemiology [2,12–17]. Increased intrinsic virulence of some GAS types, particularly types M1 and M3, has been reported [16,18–23]. These M-types frequently produce streptococcal pyrogenic exotoxins, which have the capacity to non-specifically

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activate a large subset of T-cells, resulting in a massive release of cytokines [24]. This mechanism has been implicated in the pathogenesis of severe complications such as TSS [25].

Only a few population-based prospective epidemiological studies [4,16,18,20,26–28] have addressed the long-term dynamics of invasive GAS disease and the contribution of 'virulent' M-types over time. The present study analysed data obtained prospectively from a laboratory-based nationwide surveillance system for invasive GAS infections that has been ongoing for a period of 10 years in The Netherlands. Changes in the incidence and the relative contributions of different M-types are addressed, as well as the temporal dynamics of the population affected by invasive GAS disease.

MATERIALS AND METHODS

Surveillance

A nationwide laboratory-based surveillance system for invasive GAS infections in The Netherlands was formally organised with all regional public health laboratories (RPHLs) from May 1994. There are 16 RPHLs in The Netherlands, covering 50% of the population. Financial support was provided to RPHLs to enable submission of every GAS isolate from a normally sterile body site, together with accompanying data on the source of the isolate and the age, sex and place of residence of the patient. Weekly reports on the number (or absence) of GAS isolates obtained from normally sterile sites were also submitted. This active surveillance system for invasive GAS disease in The Netherlands (average population during the study period was 15 729 704) remained in place until December 2003.

Invasive GAS disease includes definite and probable cases [29]. Definite cases were defined as the isolation of GAS from a normally sterile site in conjunction with clinical symptoms of invasive bacterial disease. GAS isolation from a normally non-sterile site in clinical association with invasive bacterial disease was considered to be probable invasive GAS disease, but was not included in this analysis. The study design of only counting GAS isolates cultured from a normally sterile site results in an underestimation of the incidence of invasive GAS disease. The degree of underestimation was estimated using data from a nationwide surveillance of invasive GAS disease, which included clinical evaluation, that was conducted in The Netherlands in the early 1990s [30]. In this previous study, 34% of patients with clinically invasive GAS disease had GAS isolated from a non-sterile, unknown or indeterminate site. These probable cases of invasive GAS disease represented the limited sensitivity of the 'microbiologically invasive' criterion, which had a specificity of 100%, but a sensitivity of only 66% [30]. The previous study received 50% of isolates from RPHLs, in accordance with the estimated coverage of the Dutch population by RPHLs in the present study. Therefore, calculation of the incidence of invasive GAS disease in The Netherlands from the present sentinel laboratory surveillance included a correction for the limited sensitivity (× 100/66), as well as a correction for the incomplete coverage of the population by RPHLs (× 100/50).

Definition of invasiveness

Invasiveness was defined solely on microbiological criteria, i.e., the source of the isolate. GAS isolates obtained from blood, cerebrospinal, pleural, peritoneal or joint fluid, and aspirates or biopsy materials from deep, normally sterile tissues (e.g., lung, kidney, bone marrow, uterus) were included. National coverage of the RPHLs over the study period was constant, and it was assumed that the sensitivity of the microbiological definition of invasiveness (based on the period 1992–1996, when all cases were also evaluated clinically) was constant throughout the study period.

Identification and typing

Isolates sent to the National Institute of Public Health (RIVM) were confirmed as GAS following bacitracin susceptibility and latex agglutination tests (Streptex; Wellcome, Dartford, UK). All GAS isolates were subjected to M-genotyping [31], thereby allowing a direct comparison with the internationally applied M-serotyping system.

Statistical analysis

Differences in group proportions were assessed by the chi-square or Fisher's exact test, with p values < 0.05 considered significant.

RESULTS

Incidence

From May 1994 to December 2003, the RIVM received microbiologically invasive isolates of GAS from 1504 patients. Most invasive isolates were from blood (1047/1504, 70%), with other isolation sites or specimens including aspirates of abscesses, intra-operative or post-mortem swabs or tissue (118/1504, 8%), respiratory tract (114/1504, 8%), synovial fluid (69/1504, 5%), perineal or genital tract (47/1504, 3%), cerebrospinal fluid (38/1504, 2%) and others (71/1504, 5%). The absolute number of cases obtained/month during the period May 1994 to January 2004 is shown in Fig. 1. There was a clear seasonal pattern in invasive streptococcal infections, with a low incidence at the end of each year, and with most cases occurring in late winter and spring. The estimated annual incidence from 1995 until 2003 is shown in Fig. 2. The estimated incidence was highest in 1996 (4.0/100 000 individuals/year), and also relatively high in 2002 (3.1/100 000 individuals/year), with a lowest estimated incidence of 2.0/100 000 individuals/year in 1999.

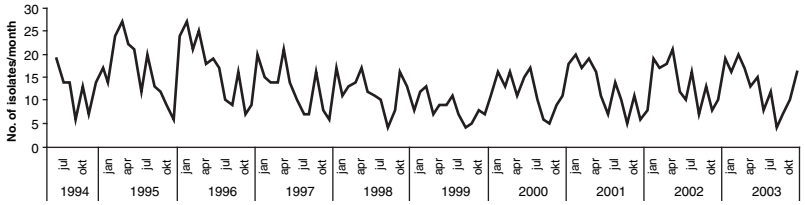

Fig. 1. Monthly distribution (observed absolute number of cases/month) of invasive group A streptococcal disease in The Netherlands (May 1994 to December 2003).

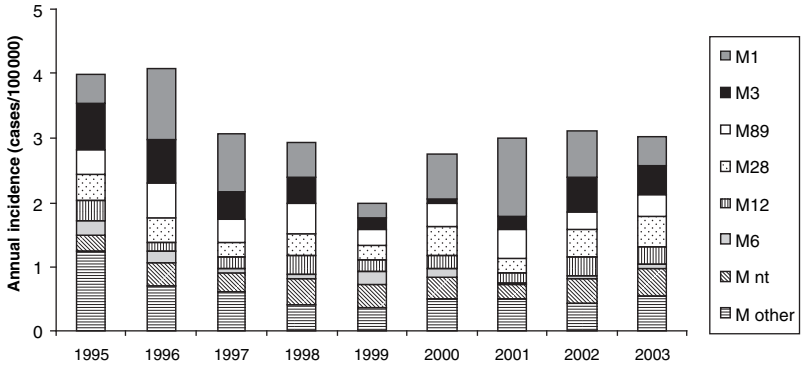

Fig. 2. Annual incidence (cases/100 000 individuals/year) of invasive group A streptococcal disease and the proportion of M1, M3, M89, M28, M12, M6, non-typeable and other M-types in The Netherlands (1995–2003).

M-types

Twenty-eight different M-types were identified, of which the most frequent were M1 (339/1504, 23%), M3 (187/1504, 12%), M89 (174/1504, 12%), M28 (164/1504, 11%), M12 (109/1504, 7%) and M6 (55/1504, 4%). Other M-types constituted 19% (292/1504) of the isolates, but although all isolates yielded an *emm* amplicon, a further 184/1504 (12%) did not hybridise with one of the M-type-specific probes (i.e., they were M-non-typeable). There was considerable variation in the relative prevalence of the predominant M-types during the study period. Thus, the proportion of M1 invasive isolates varied (Fig. 2) from 11% to 12% in 1995 (23/197) and 1999 (11/100) to 40% in 2001 (62/154). M3 isolates were rare in 2000, but comprised almost 20% of all invasive isolates in 1995 (35/197) and 2002 (27/160). The relative proportions of M1 and M3 combined correlated with the overall annual incidence (Fig. 2). Among the predominant M-types, M1, M3 and M6 showed the highest fluctuations in their relative annual contributions (Fig. 2).

Age/sex distribution of invasive GAS isolates

The date of birth was known for all cases in the present study. On the basis of a previous report [30] that described three age groups in relation to the risk of contracting invasive GAS infections, Fig. 3 shows the relative proportions of the age groups 0–20, 21–55 or ≥56 years. The group aged ≥56 years constituted 39% of all invasive cases in 1995 (76/197), increasing to >50% in 2002 (82/160) and 2003 (80/157). The relative contribution of the youngest group (aged <20 years) decreased from 19% (38/197) in 1995 to 8% (13/157) in 2003. Fig. 4 indicates that the peak in incidence for patients aged 30–34 years did not vary significantly during the 10-year surveillance period. This was the case for both men and women (results not shown).

DISCUSSION

The data from this study, obtained during a decade of nationwide laboratory-based surveillance of invasive GAS disease, indicated that the incidence of disease was highest in 1995 and 1996 (4.0/100 000 individuals/year), with a similar level almost being reached again in 2002. The lowest incidence was in 1999 (2.0/100 000 individuals/year). Sharkawy *et al.* [26] reported a nearly two-fold increase in the incidence of invasive GAS infections during a 5-year surveillance period in Ontario, Canada. The highest incidence in 1995 was similar to that reported from the USA and Israel [27,32], while the lowest incidence in the present study was similar to that reported from Ontario [18] and Sweden [13].

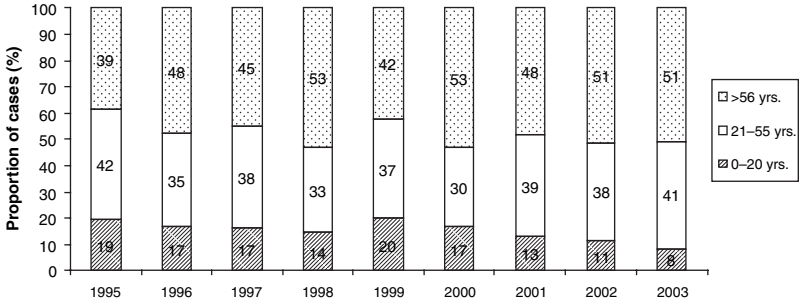

Fig. 3. Contribution of different age groups (0–20 years, 21–55 years and ≥56 years) to all cases of invasive group A streptococcal disease (1995–2003).

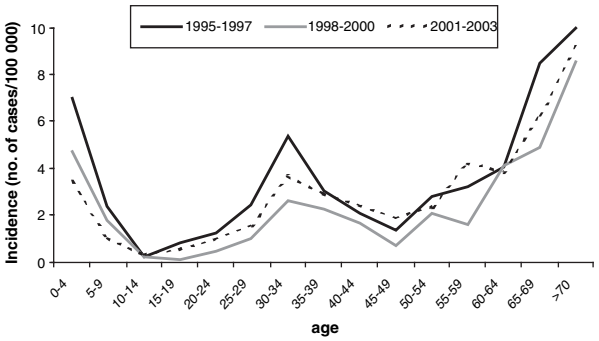

Fig. 4. Age distribution of cases of invasive group A streptococcal disease in the periods 1995–1997, 1998–2000 and 2001–2003.

For incidence calculations, it was necessary to make corrections for the national coverage of participating RPHLs, and for the fact that only isolates obtained from normally sterile sites were included. No significant changes occurred in the national coverage of RPHLs during the study period, and a previous study from 1992 to 1996 found that a constant proportion of 66% of all cases of invasive GAS disease were associated with isolation of the bacterium from a sterile site [30]. This sensitivity level was extrapolated for the present study, but it is not absolutely certain that it has remained constant over time.

Some studies, either historical [33] or hospital-based [34], have described cyclical peaks in the incidence of invasive GAS disease every 3–4 years. This was not observed in the present study, but in accordance with other reports [13,18,20,21], a clear seasonal pattern was noticed, with a sharp increase in incidence in late winter and a low incidence in summer, during the 10 years of the study.

It has been reported previously that changes in the incidence of infections associated with M1 isolates is the main explanation for changes in the overall incidence of invasive GAS disease [13,35]. The present study found that the contribution of M1 isolates, and of M1 and M3 isolates combined, correlated with the overall annual incidence. These two M-types are associated particularly with TSS and fatality [36]. Therefore, cases of GAS disease may have a more severe course during periods with a high overall incidence of invasive GAS disease.

The overall prevalence of M1 isolates was 23%. Other M-types identified frequently were M3 (12%), M89 (12%), M28 (11%), M12 (7%) and M6 (4%). In total, 12% of all isolates were M-non-typeable, which is consistent with other reports [37,38]. The high prevalence of M1 isolates was expected following the results of other studies. However, in a study conducted in the 1980s by the UK Central Public Health Laboratory, the prevalence of M1 isolates was much lower (10.4%) [38], although almost half of all isolates originated from throat swabs. Davies *et al.* [18] reported prevalences of 24% for M1, 7.4% for M12, 6.5% for M4, 6.2% for M28, and 5.8% for M3, while O'Brien *et al.* [20] found that these M-types accounted for 20.8%, 7.6%, 4.1%, 9.2% and 7.1%, respectively, of invasive isolates. Tyrell *et al.* [37] reported that M1 isolates accounted for 28.2% of all blood culture isolates, and argued that this much higher proportion, compared to the UK, might be caused either by geographical reasons or by the fact that the UK study included a large number of non-invasive isolates. In a previous study in The Netherlands [19], it was found that M1 and M3 isolates were over-represented among isolates associated with TSS and mortality, supporting the hypothesis that these serotypes have enhanced virulence [19]. Furthermore, a separate nationwide study conducted in The Netherlands in 1994–1995 [39], investigating pharyngeal carriage of GAS, showed a much lower proportion (4.5%) of M1 isolates, thereby providing evidence for an association of this

serotype with invasiveness. Therefore, the inclusion of non-invasive isolates in some studies might provide an explanation for a lower incidence of M1 isolates. However, the dynamics of M1 isolates over time should be emphasised. In the present study, involving a clearly defined geographical region, the yearly proportion of M1 isolates among all invasive isolates varied from 10% to 40%, with no evidence for the occurrence of local outbreaks. This hampers any comparison of the proportions of M1 isolates in different geographical regions over different time periods.

The proportion of any particular M-type among all invasive isolates reflects the prevalence of that serotype in the community, its invasiveness and the M-specific immunity in the population [20]. However, contemporaneous data regarding all non-invasive isolates circulating in the community is lacking. Therefore, it is not clear whether the observed fluctuations in the incidence of invasive infections with M1 and M3 isolates reflect the dynamics in the community or relative alterations in invasiveness or population immunity. Of the predominant M-types, M1, M3 and M6 isolates showed the most conspicuous fluctuations. Notably, these are the M-types that were found previously to be highly clonal in nature [36].

A high incidence of invasive infections in the elderly and youngest age groups is well-recognised [4,18,20,21,26,27,35,37,40]. In the present study, both men and women in the middle-aged category also had an increased incidence of invasive GAS disease. Whether this is associated with (selective) decreased immunity in the middle-aged group [41] or re-exposure through household contacts is unclear. However, it does not appear to be a cohort phenomenon, as the peak in the middle-aged group did not shift correspondingly during a decade of surveillance. Previous studies have shown that immunity to proteins and toxins that attach to the cell wall may play a role in protection against severe invasive disease [10]. Furthermore, a lack of protective anti-toxin antibodies has been found to be age-dependent [11,29]. The present study provides epidemiological support that this is specific for an age group rather than a cohort. However, there was a change in the relative contributions of the different age groups to all invasive GAS infections, in that the group aged 0–20 years contributed progressively less, while the group aged ≥56 years gradually contributed more. This change was not paralleled by any significant alterations in the population age distribution during the decade of the study.

In summary, the epidemiology of invasive GAS disease and the contribution of individual M-types evolve over time. Considering the overall dynamics of the various M-types, and the possibility of the appearance of new serotypes [2,12–17], continued monitoring of the distribution of different serotypes is necessary. New M-types, or virulent clones within a serotype, might appear and, with little specific immunity in the population, gain predominance. Population-based surveillance provides data for monitoring incidence trends and identifying the importance of particular virulent strains, thus providing a rational basis for disease control measures and future vaccine development.

REFERENCES

1. Efstratiou A. Group A streptococci in the 1990s. *J Antimicrob Chemother* 2000; **45**(suppl): 3–12.
2. Holm SE, Norrby A, Bergholm AM, Norgren M. Aspects of pathogenesis of serious group A streptococcal infections in Sweden, 1988–1989. *J Infect Dis* 1992; **166**: 31–37.
3. Carapetis J, Robins-Browne R, Martin D, Shelby-James T, Hogg G. Increasing severity of invasive group A streptococcal disease in Australia: clinical and molecular epidemiological features and identification of a new virulent M-nontypeable clone. *Clin Infect Dis* 1995; **21**: 1220–1227.
4. Kiska DL, Thiede B, Caracciolo J *et al*. Invasive group A streptococcal infections in North Carolina: epidemiology, clinical features, and genetic and serotype analysis of causative organisms. *J Infect Dis* 1997; **176**: 992–1000.
5. Bisno AL. Group A streptococcal infections and acute rheumatic fever. *N Engl J Med* 1991; **325**: 783–793.
6. Kaul R, McGeer A, Low DE, Green K, Schwartz B. Population-based surveillance for group A streptococcal necrotizing fasciitis: clinical features, prognostic indicators, and microbiologic analysis of seventy-seven cases. Ontario Group A Streptococcal Study. *Am J Med* 1997; **103**: 18–24.
7. Norrby-Teglund A, Chatellier S, Low DE, McGeer A, Green K, Kotb M. Host variation in cytokine responses to superantigens determine the severity of invasive group A streptococcal infection. *Eur J Immunol* 2000; **30**: 3247–3255.
8. Norrby-Teglund A, Kotb M. Host–microbe interactions in the pathogenesis of invasive group A streptococcal infections. *J Med Microbiol* 2000; **49**: 849–852.
9. Kotb M, Norrby-Teglund A, McGeer A *et al*. An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. *Nat Med* 2002; **8**: 1398–1404.
10. Akesson P, Rasmussen M, Mascini E *et al*. Low antibody levels against cell wall-attached proteins of *Streptococcus*

pyogenes predispose for severe invasive disease. *J Infect Dis* 2004; **189**: 797–804.
11. Mascini EM, Jansze M, Schellekens JF *et al*. Invasive group A streptococcal disease in the Netherlands: evidence for a protective role of anti-exotoxin A antibodies. *J Infect Dis* 2000; **181**: 631–638.
12. Colman G, Tanna A, Efstratiou A, Gaworzewska ET. The serotypes of *Streptococcus pyogenes* present in Britain during 1980–1990 and their association with disease. *J Med Microbiol* 1993; **39**: 165–178.
13. Stromberg A, Romanus V, Burman LG. Outbreak of group A streptococcal bacteremia in Sweden: an epidemiologic and clinical study. *J Infect Dis* 1991; **164**: 595–598.
14. Schwartz B, Facklam RR, Breiman RF. Changing epidemiology of group A streptococcal infection in the USA. *Lancet* 1990; **336**: 1167–1171.
15. Johnson DR, Stevens DL, Kaplan EL. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. *J Infect Dis* 1992; **166**: 374–382.
16. Hoge CW, Schwartz B, Talkington DF, Breiman RF, MacNeill EM, Englender SJ. The changing epidemiology of invasive group A streptococcal infections and the emergence of streptococcal toxic shock-like syndrome. A retrospective population-based study. *JAMA* 1993; **269**: 384–389.
17. Musser JM, Kapur V, Kanjilal S *et al*. Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of *Streptococcus pyogenes* expressing allelic variants of pyrogenic exotoxin A (Scarlet fever toxin). *J Infect Dis* 1993; **167**: 337–346.
18. Davies HD, McGeer A, Schwartz B *et al*. Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. *N Engl J Med* 1996; **335**: 547–554.
19. Schellekens JF, Schouls LM, van Pelt W, Esveld M, van Leeuwen WJ. Group A streptococci: a change in virulence? *Neth J Med* 1998; **52**: 209–217.
20. O'Brien KL, Beall B, Barrett NL *et al*. Epidemiology of invasive group A streptococcus disease in the United States, 1995–1999. *Clin Infect Dis* 2002; **35**: 268–276.
21. Svensson N, Oberg S, Henriques B *et al*. Invasive group A streptococcal infections in Sweden in 1994 and 1995: epidemiology and clinical spectrum. *Scand J Infect Dis* 2000; **32**: 609–614.
22. Cone LA, Woodard DR, Schlievert PM, Tomory GS. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. *N Engl J Med* 1987; **317**: 146–149.
23. Stevens DL, Tanner MH, Winship J *et al*. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N Engl J Med* 1989; **321**: 1–7.
24. Abe J, Forrester J, Nakahara T, Lafferty JA, Kotzin BL, Leung DY. Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. *J Immunol* 1991; **146**: 3747–3750.
25. McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. *Ann Rev Microbiol* 2001; **55**: 77–104.
26. Sharkawy A, Low DE, Saginur R *et al*. Severe group A streptococcal soft-tissue infections in Ontario: 1992–1996. *Clin Infect Dis* 2002; **34**: 454–460.
27. Moses AE, Goldberg S, Korenman Z, Ravins M, Hanski E, Shapiro M. Invasive group A streptococcal infections, Israel. *Emerg Infect Dis* 2002; **8**: 421–426.
28. Ikebe T, Murai N, Endo M *et al*. Changing prevalent T serotypes and *emm* genotypes of *Streptococcus pyogenes* isolates from streptococcal toxic shock-like syndrome (TSLS) patients in Japan. *Epidemiol Infect* 2003; **130**: 569–572.
29. The Working Group on Severe Streptococcal Infections. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. *JAMA* 1993; **269**: 390–391.
30. Vlaminckx B, Van Pelt W, Schouls L *et al*. Epidemiological features of invasive and noninvasive group A streptococcal disease in the Netherlands, 1992–1996. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 434–444.
31. Kaufhold A, Podbielski A, Baumgarten G, Blokpoel M, Top J, Schouls L. Rapid typing of group A streptococci by the use of DNA amplification and non-radioactive allele-specific oligonucleotide probes. *FEMS Microbiol Lett* 1994; **119**: 19–25.
32. Anonymous. Prevention of invasive group A streptococcal disease among household contacts of case patients and among postpartum and postsurgical patients: recommendations from the Centers for Disease Control and Prevention. *Clin Infect Dis* 2002; **35**: 950–959.
33. Krause RM. Dynamics of emergence. *J Infect Dis* 1994; **170**: 265–271.
34. Samuels N, Yinnon AM, Rudensky B. Cyclical peaking of streptococcal infections: recent and historical trends. *J Infect Dis* 1995; **172**: 1165.
35. Eriksson BK, Andersson J, Holm SE, Norgren M. Epidemiological and clinical aspects of invasive group A streptococcal infections and the streptococcal toxic shock syndrome. *Clin Infect Dis* 1998; **27**: 1428–1436.
36. Vlaminckx BJ, Mascini EM, Schellekens J *et al*. Site-specific manifestations of invasive group A streptococcal disease: type distribution and corresponding patterns of virulence determinants. *J Clin Microbiol* 2003; **41**: 4941–4949.
37. Tyrrell GJ, Lovgren M, Forwick B, Hoe NP, Musser JM, Talbot JA. M types of group A streptococcal isolates, submitted to the National Centre for Streptococcus (Canada) from 1993 to 1999. *J Clin Microbiol* 2002; **40**: 4466–4471.
38. Gaworzewska E, Colman G. Changes in the pattern of infection caused by *Streptococcus pyogenes*. *Epidemiol Infect* 1988; **100**: 257–269.
39. Zwart S, Ruijs GJ, Sachs AP, van Leeuwen WJ, Gubbels JW, de Melker RA. Beta-haemolytic streptococci isolated from acute sore-throat patients: cause or coincidence? A case-control study in general practice. *Scand J Infect Dis* 2000; **32**: 377–384.
40. Schellekens JF, Schouls LM, van Silfhout A *et al*. The resurgence of group A streptococcal disease. *Nederlands Tijdschrift Med Microbiol* 1995; **4**: 78–83.
41. Norrby-Teglund A, Pauksens K, Holm SE, Norgren M. Relation between low capacity of human sera to inhibit streptococcal mitogens and serious manifestation of disease. *J Infect Dis* 1994; **170**: 585–591.